FELINE POLYNUCLEOTIDE VACCINE FORMULA

The present invention relates to a vaccine formula allowing the vaccination of cats against a number of pathologies. It also relates to a corresponding method of vaccination.

Associations of vaccines against certain canine viruses have already been proposed in the past.

The associations developed so far were prepared from inactivated vaccines or live vaccines and, optionally, mixtures of such vaccines. Their development poses problems of compatibility between valencies and of stability. It is indeed necessary to ensure both the compatibility between the different vaccine valencies, whether from the point of view of the different antigens used or from the point of view of the formulations themselves, especially in the case where both inactivated vaccines and live vaccines are combined. The problem of the conservation of such combined vaccines and of their safety especially in the presence of an adjuvant also exists. These vaccines are in general quite expensive.

Patent Applications WO-A-90 11092, WO-A-93 19183, WO-A-94 21797 and WO-A-95 20660 have made use of the recently developed technique of polynucleotide vaccines. It is known that these vaccines use a plasmid capable of expressing, in the host cells, the antigen inserted into the plasmid. All the routes of administration have been proposed (intraperitoneal, intravenous, intramuscular, transcutaneous, intradermal, mucosal and the like). Various vaccination means can also be used, such as DNA deposited at the surface of gold particles and projected so as to penetrate into the animals' skin (Tang et al., Nature 356, 152-154, 1992) and liquid jet injectors which make it possible to transfect at the same time the skin, the muscle, the fatty tissues and the mammary tissues (Furth et al., Analytical Biochemistry, 205, 365-368, 1992).

The polynucleotide vaccines may also use both naked DNAs and DNAs formulated, for example, inside cationic lipids or liposomes.

The invention therefore proposes to provide a multivalent vaccine formula which makes it possible to ensure vaccination against a number of canine pathogenic viruses.

Another objective of the invention is to provide such a vaccine formula combining different valencies while exhibiting all the criteria required for mutual compatibility and stability of the valencies.

Another objective of the invention is to provide such a vaccine formula which makes it possible to combine different valencies in the same vehicle.

Another objective of the invention is to provide such a vaccine which is easy and inexpensive to use.

Yet another objective of the invention is to provide a method for vaccinating cats which makes it possible to obtain protection, including multivalent protection, with a high level of efficiency and of long duration, as well as good safety.

The subject of the present invention is therefore a vaccine formula intended for cats, comprising at least three polynucleotide vaccine valencies each comprising a plasmid integrating, so as to express it in vivo in the host cells, a gene with one feline pathogen valency, these valencies being selected from those of the group consisting of feline leukaemia virus (FeLV), panleukopenia virus (FPV), infectious peritonitis virus (FIPV), coryza virus (FHV), calicivirosis virus (FCV), feline immunodeficiency virus (FIV) and possibly rabies virus (rhabdovirus), the plasmids comprising, for each valency, one or more of the genes selected from the group consisting of env and gag/pol for the feline leukaemia, VP2 for the panleukopenia, modified S (or S*) and M for the infectious peritonitis, gB and gD for the coryza, capsid for the calicivirosis, env and gag/pro for the feline immunodeficiency and G for the rabies.

Valency in the present invention is understood to mean at least one antigen providing protection

against the virus for the pathogen considered, it being possible for the valency to contain, as subvalency, one or more modified or natural genes from one or more strains of the pathogen considered.

Pathogenic agent gene is understood to mean not only the complete gene but also the various nucleotide sequences, including fragments which retain the capacity to induce a protective response. The notion of a gene covers the nucleotide sequences equivalent to those described precisely in the examples, that is to say the sequences which are different but which encode the same protein. It also covers the nucleotide sequences of other strains of the pathogen considered, which provide cross-protection or a protection specific for a strain or for a strain group. It also covers the nucleotide sequences which have been modified in order to facilitate the in vivo expression by the host animal but encoding the same protein.

Preferably, the vaccine formula according to the invention comprises the panleukopenia, coryza and calicivirosis valencies.

It will be possible to add the feline leukaemia, feline immunodeficiency and/or infectious peritonitis valencies.

As regards the coryza valency, it is preferable to use the two genes coding for gB and gD, in different plasmids or in one and the same plasmid, or to use either of these genes.

For the feline leukaemia valency, use is preferably made of the two env and gag/pol genes integrated into two different plasmids or into one and the same plasmid, or the env gene alone.

For the feline immunodeficiency valency, use will preferably be made of the two env and gag/pro genes in different plasmids or in one and the same plasmid, or only one of these genes. Still more preferably, the FeLV-A env gene and the FeLV-A and FeLV-B env genes are used.

For the infectious peritonitis valency, use is preferably made of the two M and modified S genes together in two different plasmids or in one and the same plasmid, or either of these genes. S will be modified in order to make the major facilitating epitopes inactive, preferably according to the teaching of Patent PCT/FR95/01128.

The vaccine formula according to the invention can be presented in a dose volume of between 0.1 and 3 ml and in particular between 0.5 and 1 ml.

The dose will be generally between 10 ng and 1 mg, preferably between 100 ng and 500 µg and still more preferably between 1 µg and 250 µg per plasmid type.

Use will preferably be made of naked plasmids simply placed in the vaccination vehicle which will be in general physiological saline (0.9% NaCl), ultrapure water, TE buffer and the like. All the polynucleotide vaccine forms described in the prior art can of course be used.

Each plasmid comprises a promoter capable of ensuring the expression of the gene inserted, under its control, into the host cells. This will be in general a strong eukaryotic promoter and in particular a cytomegalovirus early CMV-IE promoter of human or murine origin, or optionally of another origin such as rats, pigs and guinea pigs.

More generally, the promoter may be either of viral origin or of cellular origin. As viral promoter, there may be mentioned the SV40 virus early or late promoter or the Rous sarcoma virus LTR promoter. It may also be a promoter from the virus from which the gene is derived, for example the gene's own promoter.

As cellular promoter, there may be mentioned the promoter of a cytoskeleton gene, such as for example the desmin promoter (Bolmont et al., Journal of Submicroscopic Cytology and Pathology, 1990, 22, 117-122; and Zhenlin et al., Gene, 1989, 78, 243-254), or alternatively the actin promoter.

When several genes are present in the same plasmid, these may be presented in the same transcription unit or in two different units.

The combination of the different vaccine valencies according to the invention may be preferably achieved by mixing the polynucleotide plasmids expressing the antigen(s) of each valency, but it is also possible to envisage causing antigens of several valencies to be expressed by the same plasmid.

The subject of the invention is also monovalent vaccine formulae comprising one or more plasmids encoding one or more genes from one of the viruses above, the genes being those described above. Besides their monovalent character, these formulae may possess the characteristics stated above as regards the choice of the genes, their combinations, the composition of the plasmids, the dose volumes, the doses and the like.

The monovalent vaccine formulae may also be used (i) for the preparation of a polyvalent vaccine formula as described above, (ii) individually against the actual pathology, (iii) combined with a vaccine of another type (live or inactivated whole, recombinant, subunit) against another pathology, or (iv) as booster for a vaccine as described below.

The subject of the present invention is in fact also the use of one or more plasmids according to the invention for the manufacture of a vaccine intended to vaccinate cats first vaccinated by means of a first conventional vaccine (monovalent or multivalent) of the type in the prior art, in particular, selected from the group consisting of a live whole vaccine, an inactivated whole vaccine, a subunit vaccine, a recombinant vaccine, this first vaccine having (that is to say containing or capable of expressing) the antigen(s) encoded by the plasmid(s) or antigen(s) providing cross-protection.

Remarkably, the polynucleotide vaccine has a potent booster effect which results in an amplification of the immune response and the acquisition of a long-lasting immunity.

In general, the first-vaccination vaccines can be selected from commercial vaccines available from various veterinary vaccine producers.

The subject of the invention is also a vaccination kit grouping together a first-vaccination vaccine as described above and a vaccine formula according to the invention for the booster. It also relates to a vaccine formula according to the invention accompanied by a leaflet indicating the use of this formula as a booster for a first vaccination as described above.

The subject of the present invention is also a method for vaccinating cats, comprising the administration of an effective vaccine formula as described above. This vaccination method comprises the administration of one or more doses of the vaccine formula, it being possible for these doses to be administered in succession over a short period of time and/or in succession at widely spaced intervals.

The vaccine formulae according to the invention can be administered in the context of this method of vaccination, by the different routes of administration proposed in the prior art for polynucleotide vaccination and by means of known techniques of administration.

The subject of the invention is also the method of vaccination consisting in making a first vaccination as described above and a booster with a vaccine formula according to the invention.

In a preferred embodiment of the process according to the invention, there is administered in a first instance, to the animal, an effective dose of the vaccine of the conventional, especially inactivated, live, attenuated or recombinant, type, or alternatively a subunit vaccine, so as to provide a first vaccination, and, after a period preferably of 2 to 6 weeks, the polyvalent or monovalent vaccine according to the invention is administered.

The efficiency of presentation of the antigens to the immune system varies according to the tissues. In particular, the mucous membranes of the respiratory

tree serve as barrier to the entry of pathogens and are associated with lymphoid tissues which support local immunity. The administration of a vaccine by contact with the mucous membranes, in particular the buccal mucous membrane, the pharyngeal mucous membrane and the mucous membrane of the bronchial region is certainly of interest for vaccination against respiratory and digestive pathologies.

Consequently, the mucosal routes of administration form part of a preferred mode of administration for the invention, using in particular nebulization or spray or drinking water. It will be possible to apply the vaccine formulae and the vaccination methods according to the invention in this context.

The invention also relates to the method of preparing the vaccine formulae, namely the preparation of the valencies and mixtures thereof, as evident from this description.

The invention will now be described in greater detail with the aid of the embodiments of the invention taken with reference to the accompanying drawings.

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Sequence listing SEQ ID No.

SEQ ID No. 1: Oligonucleotide PB247
SEQ ID No. 2: Oligonucleotide PB249
SEQ ID No. 3: Oligonucleotide PB281
SEQ ID No. 4: Oligonucleotide PB282
SEQ ID No. 5: Sequence of the FeLV-B virus env gene
SEQ ID No. 6: Oligonucleotide PB283
SEQ ID No. 7: Oligonucleotide PB284
SEQ ID No. 8: Sequence of the FeLV-A virus gag/pol gene (Glasgow-1 strain)
SEQ ID No. 9: Oligonucleotide AB021
SEQ ID No. 10: Oligonucleotide AB024
SEQ ID No. 11: Oligonucleotide AB103
SEQ ID No. 12: Oligonucleotide AB112
SEQ ID No. 13: Oligonucleotide AB113
SEQ ID No. 14: Oligonucleotide AB104
SEQ ID No. 15: Oligonucleotide AB101
SEQ ID No. 16: Oligonucleotide AB102
SEQ ID No. 17: Oligonucleotide AB106
SEQ ID No. 18: Oligonucleotide AB107
SEQ ID No. 19: Oligonucleotide AB061
SEQ ID No. 20: Oligonucleotide AB064
SEQ ID No. 21: Oligonucleotide AB065
SEQ ID No. 22: Oligonucleotide AB066
SEQ ID No. 23: Oligonucleotide AB025
SEQ ID No. 24: Oligonucleotide AB026
SEQ ID No. 25: Oligonucleotide AB067
SEQ ID No. 26: Oligonucleotide AB070
SEQ ID No. 27: Oligonucleotide AB154
SEQ ID No. 28: Oligonucleotide AB155
SEQ ID No. 29: Oligonucleotide AB011
SEQ ID No. 30: Oligonucleotide AB012

EXAMPLES

Example 1: Culture of the viruses

The viruses are cultured on the appropriate cellular system until a cytopathic effect is obtained. The cellular systems to be used for each virus are well known to persons skilled in the art. Briefly, the cells sensitive to the virus used, which are cultured in Eagle's minimum essential medium (MEM medium) or another appropriate medium, are inoculated with the viral strain studied using a multiplicity of infection of 1. The infected cells are then incubated at 37°C for the time necessary for the appearance of a complete cytopathic effect (on average 36 hours).

Example 2: Extraction of the viral genomic DNAs:

After culturing, the supernatant and the lysed cells are harvested and the entire viral suspension is centrifuged at 1000 g for 10 minutes at +4°C so as to remove the cellular debris. The viral particles are then harvested by ultracentrifugation at 400,000 g for 1 hour at +4°C. The pellet is taken up in a minimum volume of buffer (10 mM Tris, 1 mM EDTA). This concentrated viral suspension is treated with proteinase K (100 µg/ml final) in the presence of sodium dodecyl sulphate (SDS) (0.5% final) for 2 hours at 37°C. The viral DNA is then extracted with a phenol/chloroform mixture and then precipitated with 2 volumes of absolute ethanol. After leaving overnight at -20°C, the DNA is centrifuged at 10,000 g for 15 minutes at +4°C. The DNA pellet is dried and then taken up in a minimum volume of sterile ultrapure water. It can then be digested with restriction enzymes.

Example 3: Isolation of the viral genomic RNAs

The RNA viruses were purified according to techniques well known to persons skilled in the art. The genomic viral RNA of each virus was then isolated using the "guanidium thiocyanate/phenol-chloroform" extraction

technique described by P. Chomczynski and N. Sacchi (Anal. Biochem., 1987, **162**, 156-159).

Example 4: Molecular biology techniques

All the constructions of plasmids were carried out using the standard molecular biology techniques described by J. Sambrook *et al.* *(Molecular Cloning: A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). All the restriction fragments used for the present invention were isolated using the "Geneclean" kit (BIO 101 Inc. La Jolla, CA).

Example 5: RT-PCR technique

Specific oligonucleotides (comprising restriction sites at their 5′ ends to facilitate the cloning of the amplified fragments) were synthesized such that they completely cover the coding regions of the genes which are to be amplified (see specific examples). The reverse transcription (RT) reaction and the polymerase chain reaction (PCR) were carried out according to standard techniques (Sambrook J. *et al.*, 1989). Each RT-PCR reaction was performed with a pair of specific amplimers and taking, as template, the viral genomic RNA extracted. The complementary DNA amplified was extracted with phenol/chloroform/isoamyl alcohol (25:24:1) before being digested with restriction enzymes.

Example 6: plasmid pVR1012

The plasmid pVR1012 (Figure No. 1) was obtained from Vical Inc., San Diego, CA, USA. Its construction has been described in J. Hartikka *et al.* (Human Gene Therapy, 1996, **7**, 1205-1217).

Example 7: Construction of the plasmid pPB179 (FeLV-A virus env gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with feline leukaemia virus (FeLV-A) (Glasgow-1 strain) genomic RNA (M. Stewart *et*

al. J. Virol. 1986. **58**. 825-834), prepared according to the technique of Example 3, and with the following oligonucleotides:

PB247 (29 mer) (SEQ ID No. 1)

5'TTTGTCGACCATGGAAAGTCCAACGCACC3'

PB249 (28 mer) (SEQ ID No. 2)

5'TTTGGATCCTCATGGTCGGTCCGGATCG3'

so as to amplify a 1947 bp fragment containing the gene encoding the Env glycoprotein from the FeLV-A virus (Glasgow-1 strain) in the form of a SalI-BamHI fragment. After purification, the RT-PCR product was digested with SalI and BamHI in order to give a 1935 bp SalI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pPB179 (6804 bp) (Figure No. 2).

Example 8: Construction of the plasmid pPB180 (FeLV-B virus env gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with feline leukaemia virus (FeLV-B subtype) genomic RNA, prepared according to the technique of Example 3, and with the following oligonucleotides:

PB281 (29 mer) (SEQ ID No. 3)

5'TTTGTCGACATGGAAGGTCCAACGCACCC3'

PB282 (32 mer) (SEQ ID No. 4)

5'TTGGATCCTCATGGTCGGTCCGGATCATATTG3'

so as to amplify a 2005 bp fragment containing the gene encoding the Env glycoprotein from the FeLV-B virus (Figure No. 3 and SEQ ID No. 5) in the form of a SalI-BamHI fragment. After purification, the RT-PCR product was digested with SalI and BamHI in order to give a 1995 bp SalI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pPB180 (6863 bp) (Figure No. 4).

Example 9: Construction of the plasmid pPB181 (FeLV gag/pol gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the feline leukaemia virus (FeLV-A subtype) (Glasgow-1 strain) genomic RNA, prepared according to the technique of Example 3, and with the following oligonucleotides:

PB283 (33 mer) (SEQ ID No. 6)

5'TTGTCGACATGTCTGGAGCCTCTAGTGGGACAG3'

PB284 (42 mer) (SEQ ID No. 7)

5'TTGGATCCTTATTTAATTACTGCAGTTCCAAGGAACTCTC3'

so as to amplify a 3049 bp fragment containing the sequence encoding the Gag protein and the 5' part of the sequence encoding the Pol protein from the FeLV-A virus (Glasgow-1 strain) (Figure No. 5 and SEQ ID No. 8) in the form of a SalI-BamHI fragment. After purification, the RT-PCR product was digested with SalI and BamHI to give a 3039 bp SalI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pPB181 (7908 bp) (Figure No. 6).

Example 10: Construction of the plasmid pAB009 (FPV VP2 gene)

A PCR reaction was carried out with the feline panleukopaenia virus (193 strain) genomic DNA (J. Martyn *et al.*, J. Gen. Virol. 1990, **71.** 2747-2753), prepared according to the technique of Example 2, and with the following oligonucleotides:

AB021 (34 mer) (SEQ ID No. 9)

5'TGCTCTAGAGCAATGAGTGATGGAAGCAGTTCAAC3'

AB024 (33 mer) (SEQ ID No. 10)

5'CGCGGATCCATTAATATAATTTTCTAGGTGCTA3'

so as to amplify a 1776 bp fragment containing the gene encoding the FPV VP2 capsid protein. After purification, the PCR product was digested with XbaI and BamHI in order to give a 1764 bp XbaI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with XbaI and BamHI, to give the plasmid pAB009 (6664 bp) (Figure No: 7).

Example 11: Construction of the plasmid pAB053 (FIPV S* gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the feline infectious peritonitis (FIP) virus (79-1146 strain) genomic RNA (R. de Groot *et al.*, J. Gen. Virol. 1987. **68**. 2639-2646), prepared according to the technique of Example 3, and with the following oligonucleotides:

AB103 (38 mer) (SEQ ID No. 11)

5'ATAAGAATGCGGCCGCATGATTGTGCTCGTAACTTGCC3'

AB112 (25 mer) (SEQ ID No. 12)

5'CGTACATGTGGAATTCCACTGGTTG3'

so as to amplify the sequence of the 5' part of the gene encoding the virus S glycoprotein in the form of an NotI-EcoRI fragment. After purification, the 492 bp RT-PCR product was digested with NotI and EcoRI in order to liberate a 467 bp NotI-EcoRI fragment (fragment A).

The plasmid pJCA089 (Patent Application PCT/FR95/01128) was digested with EcoRI and SpeI in order to liberate a 3378 bp fragment containing the central part of the gene encoding the FIP virus modified S glycoprotein (fragment B).

An RT-PCR reaction according to the technique of Example 5 was carried out with the FIP virus (79-1146 strain) genomic RNA, prepared according to the technique of Example 3, and with the following oligonucleotides:

AB113 (25 mer) (SEQ ID No. 13)

5'AGAGTTGCAACTAGTTCTGATTTTG3'

AB104 (37 mer) (SEQ ID No. 14)

5'ATAAGAATGCGGCCGCTTAGTGGACATGCACTTTTTC3'

so as to amplify the sequence of the 3' part of the gene encoding the FIP virus S glycoprotein in the form

of an SpeI-NotI fragment. After purification, the 543 bp RT-PCR product was digested with SpeI and NotI in order to liberate a 519 bp SpeI-NotI fragment (fragment C).

The fragments A, B and C were then ligated together into the vector pVR1012 (Example 6), previously digested with NotI, to give the plasmid pAB053 (9282 bp), which contains the modified S gene in the correct orientation relative to the promoter (Figure No. 8).

Example 12: Construction of the plasmid pAB052 (FIPV M gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the feline infectious peritonitis (FIP) virus (79-1146 strain) genomic RNA (H. Vennema *et al.*, Virology. 1991, **181**. 327-335), prepared according to the technique of Example 3, and with the following oligonucleotides:

AB101 (37 mer) (SEQ ID No. 15)

5'ACGCGTCGACCCACCATGAAGTACATTTTGCTAATAC3'

AB102 (36 mer) (SEQ ID No. 16)

5'CGCGGATCCTTACACCATATGTAATAATTTTTCATG3'

so as to precisely isolate the gene encoding the FIP virus M glycoprotein in the form of a SalI-BamHI fragment. After purification, the 812 bp RT-PCR product was digested with SalI and BamHI in order to liberate a 799 bp SalI-BamHI fragment. This fragment was then ligated into the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pAB052 (5668 bp) (Figure No. 9).

Example 13: Construction of the plasmid pAB056 (FIPV N gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the feline infectious peritonitis (FIP) virus (79-1146 strain) genomic RNA (H. Vennema *et al.*, Virology. 1991, **181**. 327-335), prepared according to the technique of Example 3, and with the following oligonucleotides:

AB106 (35 mer) (SEQ ID No. 17)

5'ACGCGTCGACGCCATGGCCACACAGGGACAACGCG3'

AB107 (36 mer) (SEQ ID No. 18)

5'CGCGGATCCTTAGTTCGTAACCTCATCAATCATCTC3'

so as to precisely isolate the gene encoding the FIP virus N protein in the form of a SalI-BamHI fragment. After purification, the 1156 bp RT-PCR product was digested with SalI and BamHI in order to liberate a 1143 bp SalI-BamHI fragment. This fragment was then ligated into the vector pVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pAB056 (6011 bp) (Figure No. 10).

Example 14: Construction of the plasmid pAB028 (FHV gB gene)

A PCR reaction was carried out with the feline herpesvirus (FHV-1) (C27 strain) genomic DNA (S. Spatz *et al.* Virology. 1993. **197**. 125-36) prepared according to the technique of Example 2, and with the following oligonucleotides:

AB061 (36 mer) (SEQ ID No. 19)

5'AAAACTGCAGAATCATGTCCACTCGTGGCGATCTTG3'

AB064 (40 mer) (SEQ ID No. 20)

5'ATAAGAATGCGGCCGCTTAGACAAGATTTGTTTCAGTATC3'

so as to amplify a 2856 bp fragment containing the gene encoding the FHV-1 virus gB glycoprotein in the form of a PstI-NotI fragment. After purification, the PCR product was digested with PstI and NotI to give a 2823 bp PstI-NotI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with PstI and NotI, to give the plasmid pAB028 (7720 bp) (Figure No. 11).

Example 15: Construction of the plasmid pAB029 (FHV gD gene)

A PCR reaction was carried out with the feline herpesvirus (FHV-1) (C-27 strain) genomic DNA (S. Spatz *et al.* J. Gen. Virol. 1994. **75**. 1235-1244), prepared

according to the technique of Example 2 and with the following oligonucleotides:

AB065 (36 mer) (SEQ ID No. 21)

5'AAAACTGCAGCCAATGATGACACGTCTACATTTTTG3'

AB066 (33 mer) (SEQ ID No. 22)

5'GGAAGATCTTTAAGGATGGTGAGTTGTATGTAT3'

so as to amplify the gene encoding the FHV-1 virus gD glycoprotein in the form of a PstI-BglII fragment. After purification, the 1147 bp PCR product was digested with PstI and BglII in order to isolate a 1129 bp PstI-BglII fragment. This fragment was ligated with the vector pVR1012 (Example 6), previously digested with PstI and BglII, to give the plasmid pAB029 (5982 bp) (Figure No. 12).

Example 16: Construction of the plasmid pAB010 (FCV C gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the feline calicivirus (FCV) (F9 strain) genomic RNA (M. Carter *et al.* Virology. 1992. **190**. 443-448), prepared according to the technique of Example 3, and with the following oligonucleotides:

AB025 (33 mer) (SEQ ID No. 23)

5'ACGCGTCGACGCATGTGCTCAACCTGCGCTAAC3'

AB026 (31 mer) (SEQ ID No. 24)

5'CGCGGATCCTCATAACTTAGTCATGGGACTC3'

so as to isolate the gene encoding the FCV virus capsid protein in the form of a SalI-BamHI fragment. After purification, the 2042 bp RT-PCR product was digested with SalI and BamHI in order to isolate a 2029 bp SalI-BamHI fragment. This fragment was ligated with the vector PVR1012 (Example 6), previously digested with SalI and BamHI, to give the plasmid pAB010 (6892 bp) (Figure No. 13).

Example 17: Construction of the plasmid pAB030 (FIV env gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the feline immunodeficiency virus (FIV) (Petaluma strain) genomic RNA (R. Olmsted *et al.* Proc. Natl. Acad. Sci. USA. 1989. **86.** 8088-8096), prepared according to the technique of Example 3, and with the following oligonucleotides:

AB067 (36 mer) (SEQ ID No. 25)

5'AAAACTGCAGAAGGAATGGCAGAAGGATTTGCAGCC3'

AB070 (36 mer) (SEQ ID No. 26)

5'CGCGGATCCTCATTCCTCCTCTTTTTCAGACATGCC3'

so as to amplify a 2592 bp fragment containing the gene encoding the Env glycoprotein from the FIV virus (Petaluma strain) in the form of a PstI-BamHI fragment. After purification, the RT-PCR product was digested with PstI and BamHI to give a 2575 bp PstI-BamHI fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with PstI and BamHI, to give the plasmid pAB030 (7436 bp) (Figure No. 14).

Example 18: Construction of the plasmid pAB083 (FIV gag/pro gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the feline immunodeficiency virus (FIV) (Petaluma strain) genomic RNA (R. Olmsted *et al.* Proc. Natl. Acad. Sci. USA. 1989. **86.** 8088-8096), prepared according to the technique of Example 3, and with the following oligonucleotides:

AB154 (32 mer) (SEQ ID No. 27)

5'ACGCGTCGACATGGGGAATGGACAGGGGCGAG3'

AB155 (33 mer) (SEQ ID No. 28)

5'TGAAGATCTTCACTCATCCCCTTCAGGAAGAGC3'

so as to amplify a 4635 bp fragment containing the gene encoding the Gag and Pro proteins from the FIV virus

(Petaluma strain) in the form of a SalI-BglII fragment. After purification, the RT-PCR product was digested with SalI and BglII to give a 4622 bp SalI-BglII fragment.

This fragment was ligated with the vector pVR1012 (Example 6), previously digested with SalI and BglII, to give the plasmid pAB083 (7436 bp) (Figure No. 15).

Example 19: Construction of the plasmid pAB041 (rabies virus G gene)

An RT-PCR reaction according to the technique of Example 5 was carried out with the rabies virus (ERA strain) genomic RNA (A. Anilionis *et al.* Nature. 1981. **294**. 275-278), prepared according to the technique of Example 3, and with the following oligonucleotides:

AB011 (33 mer) (SEQ ID No. 29)

5'AAAACTGCAGAGATGGTTCCTCAGGCTCTCCTG3'

AB012 (34 mer) (SEQ ID No. 30)

5'CGCGGATCCTCACAGTCTGGTCTCACCCCCACTC3'

so as to amplify a 1589 bp fragment containing the gene encoding the rabies virus G glycoprotein. After purification, the RT-PCR product was digested with PstI and BamHI to give a 1578 bp PstI-BamHI fragment. This fragment was ligated with the vector pVR1012 (Example 6), previously digested with PstI and BamHI, to give the plasmid pAB041 (6437 bp) (Figure No. 16).

Example 20: Production and purification of the plasmids

For the preparation of the plasmids intended for the vaccination of animals, any technique may be used which makes it possible to obtain a suspension of purified plasmids predominantly in the supercoiled form. These techniques are well known to persons skilled in the art. There may be mentioned in particular the alkaline lysis technique followed by two successive ultracentrifugations on a caesium chloride gradient in the presence of ethidium bromide as described in J. Sambrook *et al.* (*Molecular Cloning: A Laboratory*

Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). Reference may also be made to Patent Applications PCT WO 95/21250 and PCT WO 96/02658 which describe methods for producing, on an industrial scale, plasmids which can be used for vaccination. For the purposes of the manufacture of vaccines (see Example 17), the purified plasmids are resuspended so as to obtain solutions at a high concentration (> 2 mg/ml) which are compatible with storage. To do this the plasmids are resuspended either in ultrapure water or in TE buffer (10 mM Tris-HCl; 1 mM EDTA, pH 8.0).

Example 21: Manufacture of the associated vaccines

The various plasmids necessary for the manufacture of an associated vaccine are mixed starting with their concentrated solutions (Example 16). The mixtures are prepared such that the final concentration of each plasmid corresponds to the effective dose of each plasmid. The solutions which can be used to adjust the final concentration of the vaccine may be either a 0.9% NaCl solution, or PBS buffer.

Specific formulations such as liposomes, cationic lipids, may also be used for the manufacture of the vaccines.

Example 22: Vaccination of cats

The cats are vaccinated with doses of 10 µg, 50 µg or 250 µg per plasmid.

The injections are performed with a needle by the intramuscular route. In this case, the vaccinal doses are administered in a volume of 1 ml.

The injections can also be performed with a needle by the intradermal route. In this case, the vaccinal doses are administered in a total volume of 1 ml administered at 10 points of 0.1 ml or at 20 points of 0.05 ml. The intradermal administrations are performed after shaving the skin (thoracic flank in general) or at the level of a relatively glabrous

anatomical region, for example the inner surface of the thigh.

A liquid jet injection apparatus (with no needle) can also be used for the intradermal injections.